Observations on pulpal response to carbon dioxide laser drilling of dentine in healthy human third molars.

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Abstract: Preservation of pulpal health is the primary prerequisite for successful application of laser systems in the hard tissue management of vital teeth. The purpose of this study was to investigate the short and long-term pulpal effects to cavity preparations in healthy human teeth using carbon dioxide (CO2) laser. A total of seven, healthy, third molars that were scheduled to be removed due to space problems were used. After the laser drilling, the occlusal cavities were closed temporarily, and the teeth were extracted 7 days (n=5) and 3 months (n=2) after the operation. The specimens were fixed, decalcified, subdivided and processed for light and transmission electron microscopy. Seven days postoperatively all the five teeth that had been irradiated with the CO2 laser did not reveal any pathological changes in the pulpo-dentine complex. Three months postoperatively the two teeth that were prepared with the laser showed subtle but distinct apposition of tertiary dentine that was lined with intact odontoblasts. One of the specimens at 3 months revealed the presence of a mild, but very circumscribed, pulpal infiltration of chronic inflammatory cells subjacent to the cavity preparation. The latter is unlikely to be due to a direct effect of the laser irradiation but a possible consequence of microleakage of oral antigens and/or other tissue-irritating molecules through the temporary restoration and the remaining dentine thickness (RDT). Although these preliminary histological results suggest that the CO2 laser under investigation induced only minimal response of the dentine-pulp complex when used as a hard-tissue drilling tool, with specific energy settings, pulse duration within thermal relaxation time and emitting radiations at 9.6 microm of wavelength, larger clinical trials involving various types of teeth are necessary to reach definite conclusions for large-scale clinical application of the laser device.

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ORIGINAL ARTICLE

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Keywords Cavity-preparation · Dental CO_2 lasers · Histology · Pulpal effects · Tissue ablation

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Introduction

The application of the physical principle of 'stimulated emission of radiation' [1] to the visible segment of the electromagnetic spectrum [2] resulted in *light amplification by stimulated emission of radiation* (laser). Shortly thereafter, ruby laser, the first device using laser energy, was developed [3] and tried in dentistry [4–7]. Owing to several reasons there had been great interest among dental clinicians and researchers for lasers, with the ultimate goal of replacing the hand drill with a dental laser device. The history, development and application of lasers in medicine and dentistry have been very well reviewed [8–12]. Several laser systems, such as argon (Ar), carbon dioxide (CO_2), helium–neon (He–Ne), neodymnium:yttrium–aluminium–garnet (Nd:YAG), erbium (Er):YAG, holmium (Ho):YAG, and so on, became commercially available for therapeutic and research purposes. However, owing to the enormous and unregulated generation of heat during the irradiation, the early application of lasers on teeth resulted in severe damage of the hard tissues and irreversible necrosis of the tooth pulp. While there has been considerable progress in the commercial, strategic [13] and general medical applications of laser, its utility for dental hard tissue management remained a distant dream. Reasons for this include inadequate knowledge of the physical properties of the laser, the complex interaction of dental hard tissues with the laser energy and the absence of stringent physical standards for safe therapeutic application of the

novel devices. This resulted in certain pessimism about the future of lasers in dentistry.

Notwithstanding the initial setbacks, the sustained professional demands for an ideal laser instrument for hard tissue operations led to significant advances in laser research. The breakthrough came with the identification of appropriate laser wavelengths that cause selective damage to tissue components by selective absorption of pulsed radiation (photothermolysis [14]) of low energy, pulse duration within thermal relaxation time and other safety parameters to be set in such systems. In addition, integrated water cooling is also provided in the units to minimise heat distribution [15]. Consequently, the availability of a new generation of laser devices [16–18] with improved hard tissue cutting efficiency but diminished heat distribution for application on hard tissues has become a reality. The choice has been narrowed down to the CO_2 and Er:YAG lasers [17] operating at wavelengths of 9.6 μm and 2.94 μm, respectively [19].

It is absolutely essential that the histological effects of a laser system on tooth pulp be studied before any large-scale routine application of the device on the general public can be envisaged. There have been a few animal [20–22] and human [23, 24] studies on the Er:YAG laser, with positive outcomes. Our own study, published recently, [24] showed excellent pulp preserving quality of a Er:YAG laser operating at 2.94 μm wavelength. However, the pulpal effects of CO_2 laser drilling of coronal dentine were investigated only in animals, mostly with devices irradiating at 10.6 μm wavelength and long pulse duration, which cause severe thermal injury to tissues [25, 26]. To our knowledge, histological and fine structural investigations on the pulpal effect of CO_2 laser drilling of human teeth do not seem to exist. Therefore, the aim of this trial was to investigate the short and long-term pulpal effects of CO_2 laser irradiation of coronal dentine in healthy human teeth.

Materials and methods

Patients and teeth

A total of seven, clinically healthy, third molars, scheduled for removal due to space problems, were used. The teeth originated from five patients who were treated in accordance with the Helsinki declaration and with the permission of the local ethics committee. The informed consent of each patient was obtained after the clinical procedures had been explained, together with the risks involved and the benefits, and after all questions raised by the patients had been clarified. The clinical status of the seven laser-drilled teeth was determined by a combination of physical examination, radiography and tooth sensitivity test. Physical and radiographic examinations revealed that all the teeth were caries free and asymptomatic. Pulp sensitivity was determined by the application of a dry ice stick (CO_2) to the cervical area of the involved tooth. All the seven teeth were positive for the cold thermal sensitivity test. A caries free, asymptomatic and cold-sensitive tooth was assumed to be clinically healthy, with a vital pulp.

CO_2 Laser

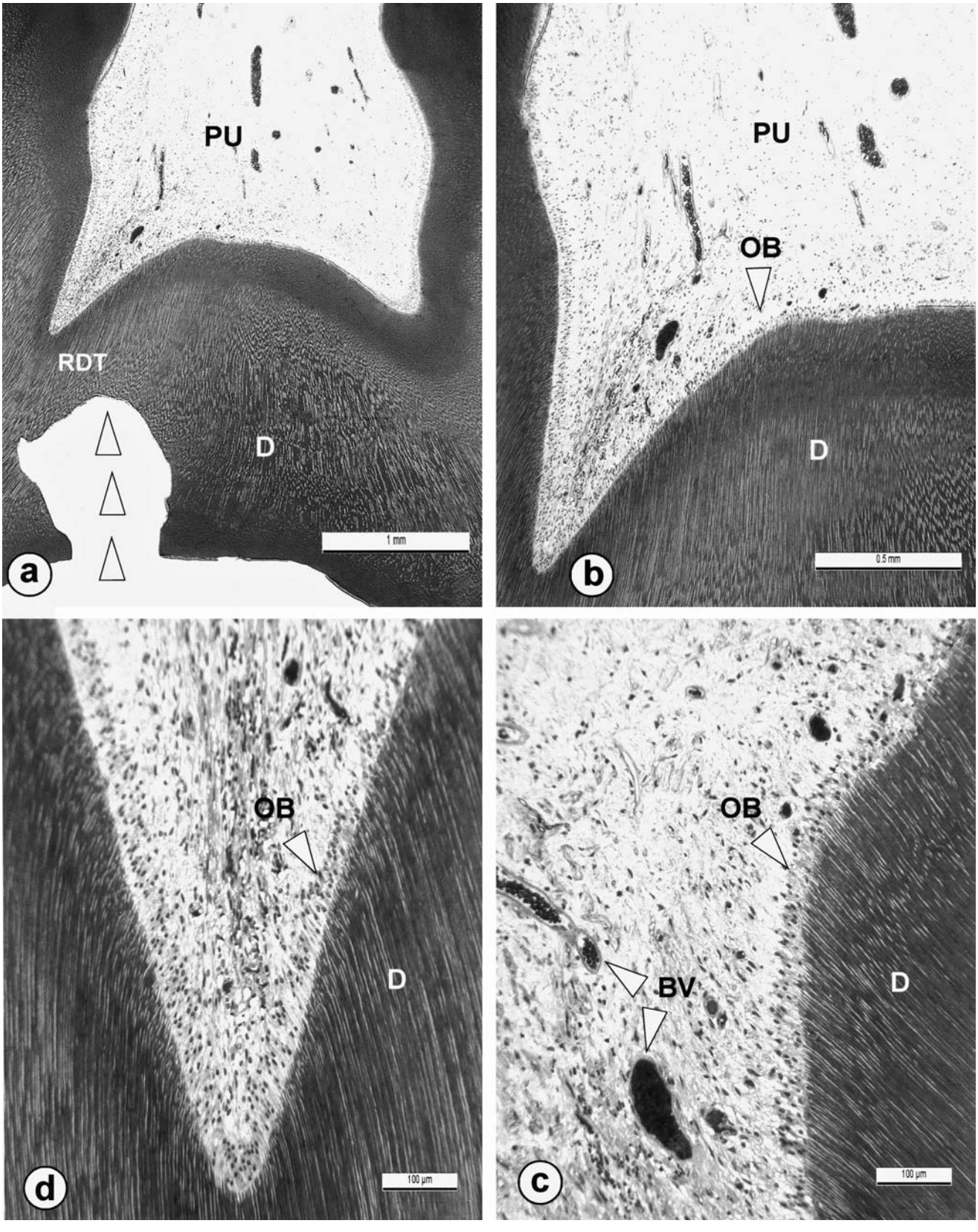
A super-pulsed CO_2 laser (ESE-Sharplan, Sharplan Laser Industries, Tel Aviv, Israel), with an emitting wavelength of 9.6 μm, was used. Other laser parameters included a pulse width of 60 μs, pulse energy of 40 mJ, and a repetition rate of 100 Hz. A built-in water-cooling system, started 3 s before the onset of irradiation and stopped 3 s after its termination, sprayed 15 ml of cooling water per min. An integrated scanner system allowed controlled ablation of the substrate for a 2.5-mm-wide area, which was delivered with a series of single pulses of 300 μm spot size in a hexagonal circle.

Tissue processing

Immediately after being extracted the teeth were fixed by immersion in half-strength Karnovsky's fixative for several days [27]. Thereafter, the specimens were decalcified for several weeks in solutions containing 0.25 mol/l ethylene diamine tetra-acetic acid (Titriplex-III, Merck) and 4% glutaraldehyde. The demineralised teeth were subdivided into approximately 0.3–0.5 mm-thick slices (in either the mesio-distal or bucco-lingual plane), post-fixed in 1.33% osmium tetroxide (OsO_4), dehydrated in ascending grades of ethanol and embedded in Epon [28]. From each Epon block 1–2 μm-thick survey sections and, from selected blocks, serial sections, were prepared, with glass or histodiamond knives (Diatome AG, Biel, Switzerland) and the Reichert Ultracut E microtome (Leica, Glattbrugg, Switzerland). The sections were stained in periodic acid–Schiff (PAS) and methylene blue-Azur II and photomicrographed with a Dialux 20 photomicroscope (Leica, Glattbrugg, Switzerland) equipped with the digital camera Progress C14 (Jenoptik, Eching, Germany) and a digital imaging system (ImageAccess, Imagic, Glattbrugg, Switzerland). The sections were studied thoroughly in a light microscope for any physical and/or pathobiological changes in the dentine and pulp. Thereafter, suitable areas in the Epon blocks were determined for ultra-sectioning. Such selected tissue sites were target-trimmed and thin-sectioned with the Reichert Ultracut E microtome (Leica). The thin sections were double-contrasted with lead and uranium salts [29, 30] and examined with a Philips EM400T transmission electron microscope (TEM).

Results

In all cases in both the short and long-term observation periods the postoperative clinical condition of the teeth



presented no complications. Pain and other signs of inflammation or infections were not evinced or detected throughout the observation periods. The depth of the laser drilling could not be precisely regulated, and the coronal pulps of the teeth under investigation were separated from the bottom of the cavity preparations by

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Fig. 1 A photomicrograph (**a**) of the left maxillary third molar (tooth index 28) of a female patient, showing dentine cavity preparation (*arrowheads*) with CO_2 laser 7 days after the operation. Note the remaining RDT of 480 μm thickness. **b** Magnification of part of the RDT and pulp subjacent to the cavity. The odontoblastic layer (*OB*; *arrowhead*) and tip of the pulpal horn in **b** are further enlarged in **c** and **d**, respectively. Note the intact pulp (*PU*), odontoblasts (*OB*) and dentine (*D*) subjacent to the cavity preparation (*arrowheads* in **a**) with 480 μm of RDT in **a**. Original magnifications **a** ×25, **b** ×52, **c** and **d** ×130

some remaining dentine of varying thickness. The seven laser-irradiated teeth had an average remaining dentine thickness (RDT) of 1.1 mm (range 0.5–1.5 mm).

One-week specimens

All five teeth in the short-term group did not reveal any histologically observable signs of dentine-pulp response to the laser cutting of dentine. The architecture of the RDT associated with the cavity preparation and the peripheral pulp appeared normal (Fig. 1a), with intact tubular dentine, predentine and odontoblasts. The soft tissue subjacent to the RDT revealed the typical histological appearance of healthy pulp, with pseudostratified odontoblasts and subodontoblastic pulpal components (Fig. 1b, c, d).

Twelve-week specimens

The two teeth under observation revealed the formation of a subtle but distinct layer of tertiary dentine on the pulpal aspect of the RDT (Fig. 2). In methylene blue/PAS-stained Epon-embedded sections the tertiary dentine appeared similar in colour to predentine on either side but appeared wider (Fig. 2b, c) and contained more or less similar numbers of dentinal tubules (Fig. 2c). The rest of the peripheral pulp was histologically normal in one of the two specimens.

However, in the remaining specimen, the peripheral pulp subjacent to the cavity preparation revealed a small circumscribed area of mild infiltration with chronic inflammatory cells (Fig. 2c, d). In the TEM (Fig. 3) the infiltrate consisted of lymphocytes, plasma cells and macrophages, with occasional neutrophilic granulocytes (Fig. 3 insets).

Discussion

Since the first application of a laser device in dentistry [4], commercial availability of a suitable dental laser for safe and efficient cavity preparations has been a cherished goal in dental medicine. The primary prerequisite for such an instrument is its ability to preserve the integrity of the tooth pulp. This study provides histological evidence that the CO_2 laser under investigation caused no damaging thermal effect of the pulp and induced only very subtle defence responses of the pulpo-dentine complex when used with the specific energy settings, pulse duration within thermal relaxation time, emitting radiations at a wavelength of 9.6 μm and with provision for water cooling.

In the 1 week postoperative specimens (short term), there were no structurally detectable signs of pulpal changes, such as vacuolar degeneration, disruption of the odontoblastic layer, haemorrhage, inflammation or necrosis, in any of the five CO_2 laser-treated teeth. There are only a few publications in the literature [22, 31–33] about the pulpal impact of CO_2 laser irradiation of coronal dentine. Earlier studies report damaging short-term dentinal and pulpal effect of the laser—a finding in discord with the results presented here. The thermal effects include charring of the cut surface of the RDT [22], disruption of predentine, loss of odontoblastic cell layer, congestion of subodontoblastic blood vessels, extravasation and vacuolar degeneration [22, 31]. It may be noted that the studies were not recent and were based on CO_2 laser devices that irradiated at 10.6 μm wavelength under non-optimal energy settings.

On the other hand, the short-term pulpal effects of the present investigation are in full agreement with those of a recent animal study [33], in which a refined CO_2 laser that emitted 22 mJ of 9.6 μm radiation in 60-μs-long pulses was used. The total absence of injurious thermal effects on the pulp was possibly due to several reasons, such as the wavelength of the laser, the characteristic interaction between the CO_2 laser and the hard tissues of the teeth, the non-injurious energy settings, low pulse energy with a pulse duration close to the thermal relaxation time and the provision of water cooling. The 9.6-μm wavelength of the laser coincides with the principal absorption band of hydoxylapatite, the major component of enamel and dentine. In addition, the water present in the enamel and dentine of healthy teeth in vivo, and the collagen of dentine also absorb the 9.6-μm wavelength energy. The water cooling of the laser system aided the prevention of heat distribution, so that the potential rise in pulpal temperature did not reach the critically injurious level of 5.5°C [34].

The pulp-protective effect of RDT may also be considered. As the depth of the laser drilling could not be precisely controlled, the coronal pulps of the laser-drilled teeth were separated by varying RDTs that ranged between 0.5 mm and 1.5 mm. To our knowledge no published data seem to exist on the pulp-protective effect of RDT in CO_2 and other laser-drilled teeth. Nevertheless, it is possible that the RDT may also have played a role in the total absence of pulpal changes in six of the seven specimens.

In general, the long-term effect of the CO_2 laser application consisted of the formation of tertiary dentine of varying thickness subjacent to the area of laser application [31–33]. In the 3 months postoperative group (long term) of the present study, the two teeth revealed a subtle but characteristic reparative/defence response of the pulpo-dentine complex by deposition of a thin layer of reactive tertiary dentine. This is fully

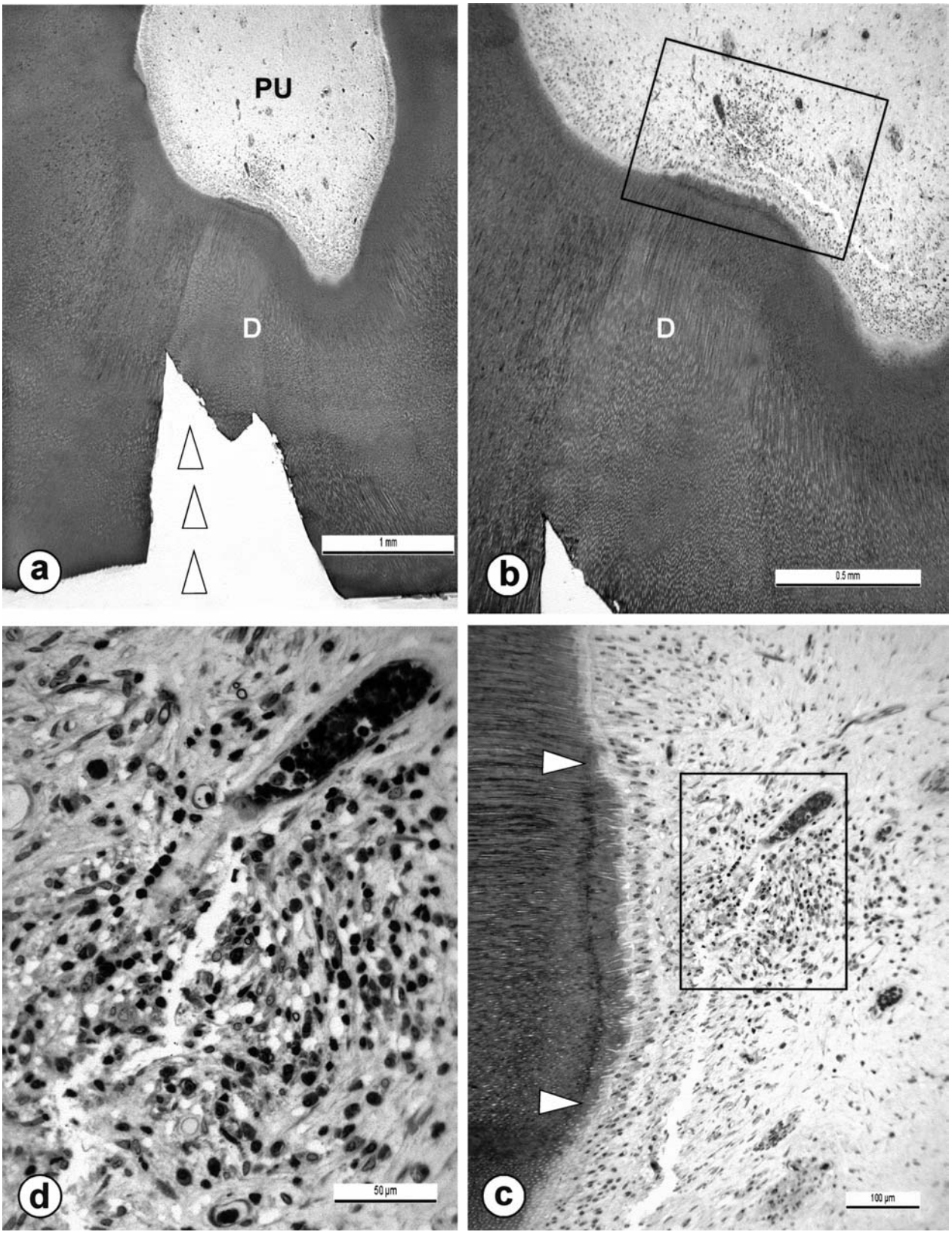

Fig. 2 A photomicrograph (**a**) of the left maxillary third molar (tooth index 28) of a male patient showing cavity preparation (*arrowheads*) in dentine with a CO_2 laser 3 months after the operation. Note the dentine had a remaining thickness of 1.2 mm beneath the cavity. The rectangular demarcated areas in **b** and **c** are magnified in **c** and **d**, respectively. Note the subtle but distinct zone of tertiary dentine (*arrowheads* in **c**) with intact odontoblastic layer. The peripheral pulp (*PU*) subjacent to the cavity preparation shows mild infiltration of chronic inflammatory cells. *D* dentine. Original magnifications **a** ×24, **b** ×52, **c** ×130, **d** ×360

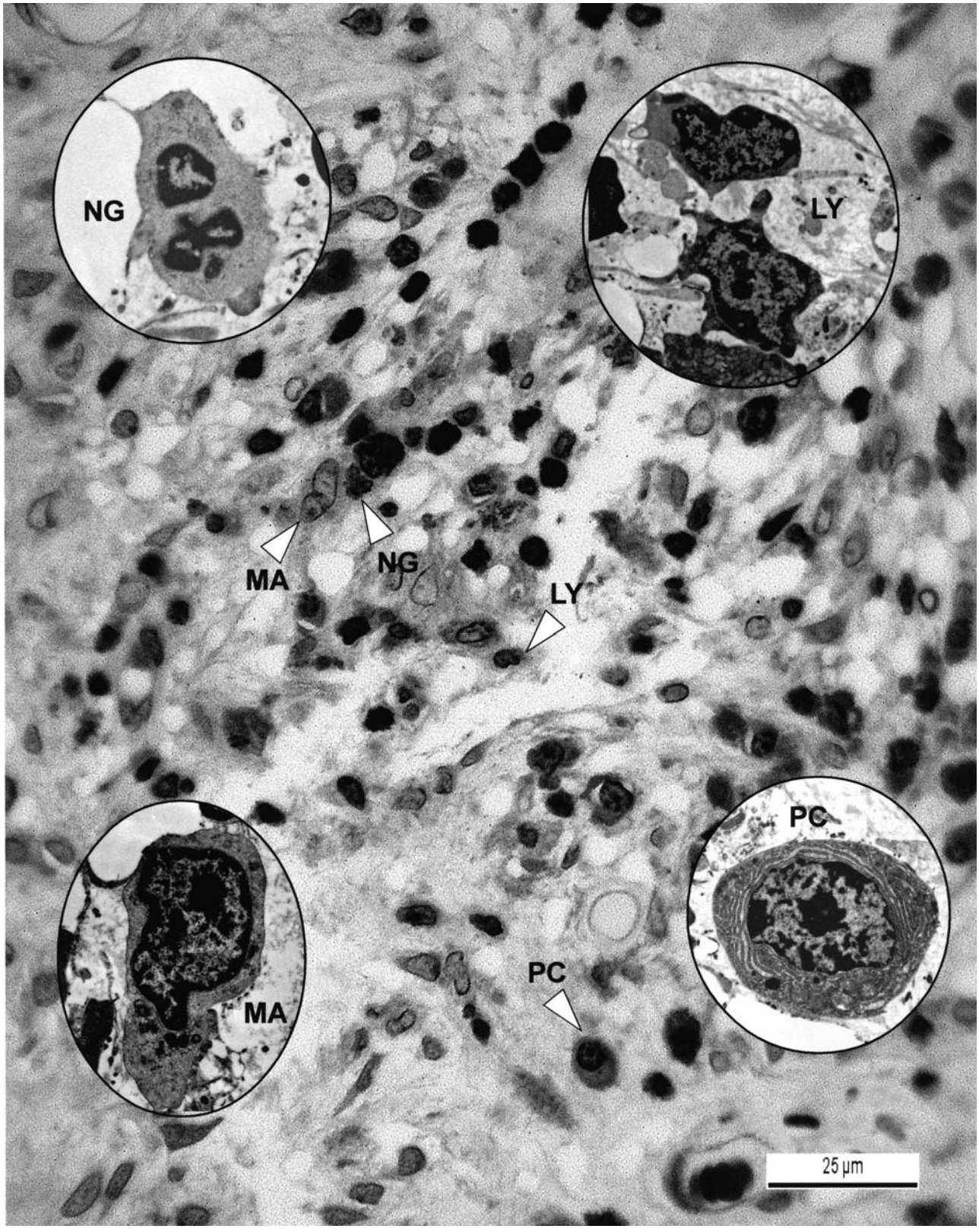

Fig. 3 A high-power light-microscopic view of the infiltrate shown in Fig. 2d. Note the numerous lymphocytes (*LY*) and plasma cells (*PC*) with occasional macrophages (*MA*) and neutrophils (*NG*) in the infiltrate. The *insets* show representative transmission electron microscopic views of a neutrophil (*upper left*), lymphocyte (*upper right*), a plasma cell (*lower right*) and macrophage (*lower left*). Original magnifications ×1,050; *lower insets* ×4,600, *upper left inset* ×7,150, *upper right inset* ×4,000

consistent with the reported formation of tertiary dentine in response to CO_2 laser drilling in other studies [31–33], even under various observation periods and energy settings. The subtlety and the normal tubular structure of the tertiary dentine formed are indicative of the absence of serious odontoblastic damage as a result of the laser irradiation of dentine. The formation of the tertiary dentine may be due to a direct effect of the laser irradiation, a response to the temporary filling material and/or a combined effect of both. It may be pointed out that the cavities prepared had to be observed for varying periods of time, which could not be done ethically in the absence of any temporary restoration so as to avoid any adverse effects of oral antigens that might diffuse through the cut dentinal tubules of the RDT. Therefore, it was not practicably possible to separate the possible effects of the laser from that, if any, of the restorative material used in this study by control experiments.

The cause of the mild, circumscribed, pulpal infiltration of chronic inflammatory cells subjacent to the cavity preparation in one tooth (Fig. 2) is unknown. The total absence of a chronic inflammatory response in any of the teeth in the 1 week of observation and also in the other replication in the longer observation period suggests that the observed pulpal reaction in one tooth may not have been due to a direct effect of the laser irradiation. The limited, circumscribed, infiltration of lymphocytes, plasma cells and macrophages in the tooth specimen is more of an indication of a mild antigenic challenge and/or of low-grade tissue irritation in the area. The offending molecules most probably might have reached the pulp (a) from the oral cavity by microleakage of the temporary filling and the porous nature of the RDT and the thin layer of cut tubular tertiary dentine and/or (b) from release from the temporary fillings that obtained access to the pulp. It may be noted that there was a total absence of any vacuolar degenerative changes or acute inflammatory cell response in any of the specimens in the shorter (1 week) observation period and also in the other replication in the longer observation period. Therefore, it is reasonable to assume that the mild, chronic, inflammatory response observed in one tooth is unlikely to be due to a direct effect of laser irradiation.

These preliminary findings, nevertheless, allow the conclusion to be drawn that coronal cavity preparations of human teeth can be done with CO_2 laser of specified settings with minimal response of the dentine-pulp complex. Larger clinical trials involving different types of teeth are required to reach definite conclusions.

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